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2. Patent application number *(The Patent Office will fill in this part)*	**9921242.5**
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	IMPERIAL CANCER RESEARCH TECHNOLOGY LIMITED Sardinia House Sardinia Street LONDON WC2A 3NL
Patents ADP number *(if you know it)*	04308243002
If the applicant is a corporate body, give the country/state of its incorporation	GB
4. Title of the invention	CANCER VACCINE AND DIAGNOSIS
5. Name of your agent *(if you have one)*	J A KEMP & CO
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	14 SOUTH SQUARE GRAY'S INN LONDON WC1R 5LX
Patents ADP number *(if you know it)*	26001
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11. I/We request the grant of a patent on the basis of this application

Signature J. A. Kemp & Co Date 8 September 1999

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CANCER VACCINE AND DIAGNOSIS

The invention relates to MHC class I restricted epitopes and T cells which can be used to prevent or treat cancer or cause immunosuppression; and to the use of the epitopes to diagnose cancer.

Cytotoxic T lymphocytes (CTL) recognise epitopes bound to MHC class I molecules on the surface of cells. Recognition of such epitopes on the surface of target cells by CTL leads to the killing of the target cells by the CTL. The epitopes which are displayed on the cell are fragments from proteins which have been processed in the class I antigen processing pathway of the cell. In this pathway proteins (generally from the cytoplasm) are broken down in the cytoplasm into small peptides. The small peptides are then transported through the endoplasmic reticulum (where they bind to the MHC molecules) to the surface of the cell.

It is not possible to predict which proteins will enter the antigen processing pathway, which fragments will be produced, or which fragments will bind to MHC molecules and be presented at the surface of the cell. Additionally it is not possible to predict which fragments T cells will recognise and whether the T cells which recognise the fragments will be protective.

The inventors have identified epitopes which can be used to induce a MHC class I restricted response which is protective against a tumour challenge. The epitopes are from the MUC1 protein, but lie outside the immunogenic variable non tandem repeat (VNTR) region. Since activated T cells express MUC1 the epitopes can also be used to induce an immune response against such T cells or be used to obtain products which are capable of targeting activated T cells. The epitopes are represented by or present in the sequences SEQ ID NO:1, 2 and 3.

The invention thus provides an agent selected from

(i) peptides that comprise a MHC class I restricted T cell epitope, the epitope being contained in or represented by SEQ ID NO:1, 2, or 3; excluding whole MUC1, (ii) analogues of peptides (i) which are capable of inhibiting the binding of the said epitope to a T cell receptor either by directly binding to the same T cell receptor or by binding to the same T cell receptor after being processed, and (iii) polynucleotides which are capable of being expressed to provide peptides (i) or analogues (ii).

The invention also provides (iv) a composition that comprises two or more different compounds, wherein each of the compounds is an agent as defined above.

The invention further provides the agent or composition for use in a method of vaccination against cancer or for use in a method of immunosuppression. The invention additionally provides a vaccine comprising the agent or composition which vaccine is capable of stimulating a MHC class I restricted T cell response directed to an epitope of the invention.

The invention provides a T cell receptor which recognises an epitope of the invention; or a fragment thereof which can recognise the epitope. The invention also provides a product that selectively binds a T cell receptor of the invention.

The invention provides a method of diagnosing cancer in a host said method comprising determining the presence or absence in the host of a MHC class I restricted T cell response to an epitope of the invention, the presence of the response indicating that the host has cancer.

MHC class I restricted T cells are discussed herein. These cells are typically CD8 T cells, but may be CD4 T cells.

The sequence of the epitope present in the peptide of the invention may be any of the sequences represented

by SEQ ID NOs 1 to 3 below or epitopes present within these sequences (such as the fragments of the sequences shown in the brackets):

SEQ ID NO:1 - FLSFHISNL (LSFHISNL)
SEQ ID NO:2 - ALGSTAPPV (LGSTAPPV)
SEQ ID NO:3 - TLAPATEPA (LAPATEPA)

In one embodiment the peptide of the invention has the same sequence as the epitope. The peptide typically comprises 1, 2, 3 or more copies of each of 1, 2 or more, or all of the above epitopes.

Typically in the peptide, 'linker' sequence may or may not separate the epitopes and/or there may or may not be additional (non-epitope) sequence at the N terminal or C terminal of the peptide. Typically the peptide comprises 1, 2, 3 or more linkers. The linkers are typically 1, 2, 3, 4 or more amino acids in length and may comprise amino acid sequence encoded by a polynucleotide sequence that comprises enzyme restriction sites or amino acids that constitute proteosomal cleavage sites. Thus in the peptide 1, 2 or more, or all of the epitopes may be contiguous with each other or separated from each other. The epitope sequences may overlap with each other. The peptide is typically 8 to 2000 amino acids in length, such as 9 to 1000, 10 to 500, 11 to 200, 12 to 100 or 15 to 50 amino acids.

The peptide may be a natural protein, a fragment thereof, a non-natural protein, or a fusion protein (typically) comprising sequence from different proteins.

The peptide may or may not comprise or be a fragment of MUC1, which fragment may or may not include the MUC1 VNTR. Thus in one embodiment such a fragment only comprises sequence from outside the VNTR. Table 1 shows a representation of the MUC1 amino acid sequence in which only a single perfect copy of the tandem repeat sequence

is shown by amino acids 129 to 148 inclusive. In one embodiment the peptide of the invention comprises fragments of the MUC1 sequence shown in Table 1, which fragments do not include any sequence from amino acids 97 to 184, e.g. fragments which do not include sequence from amino acids 90 to 190.

SEQ ID NO's 1, 2 and 3 can be seen at amino acid positions 264 to 272 (inclusive), 167 to 175 (inclusive) and 79 to 87 (inclusive) of Table 1. In one embodiment the peptide only contains MUC1 sequence that lies close to the epitopes, such as only sequence from or within positions 258 to 276, 253 to 281, 161 to 179, 156 to 184, 72 to 91 or 67 to 96.

The peptide may also comprise sequence which aids the stimulation of a CTL response directed to the epitope. Such sequence may act as adjuvant or may target the peptide to antigen presenting cells (APCs) or to compartments in the antigen processing pathway. The sequence may stimulate a T helper response, such as a Th1 response, and thus may comprise a T helper (e.g. Th1) cell epitope. The peptide may also comprise the sequence of any of the proteins mentioned herein.

The peptide may be free from modifications. In one embodiment the peptide is modified, for example by a natural post-translational modification (e.g. glycosylation) or an artificial modification. Thus sequence in the peptide may or may not comprise the modification(s) that are present when the sequence is expressed in a normal or cancer cell. The peptide may comprise the modifications that occur when it is expressed in a eukaryotic (e.g. human) or prokaryotic (e.g. E. coli) cell. In one embodiment the peptide lacks glycosylation.

The modification may provide a chemical moiety (typically by substitution of a hydrogen, e.g. the

hydrogen of a C-H bond), such as an amino, acetyl, hydroxy or halogen (e.g. fluorine) group or carbohydrate group. Typically the modification is present on the N or C terminus.

The analogue of the invention is capable of inhibiting the binding of any of the epitopes to a T cell receptor, either directly or after the analogue is processed. Therefore certain analogues of the invention can be processed to provide other analogues (that can bind the T cell receptor directly). The term 'analogue' as used below includes both of these types of analogue. The term 'processed' refers to being processed by the class I antigen presentation pathway (generally this will be hydrolysis, e.g. proteolysis).

Typically the amount of epitope which can bind the T cell receptor in the presence of the analogue is decreased. This is because the analogue is able to bind the T cell receptor and therefore competes with the epitope for binding to the T cell receptor. The binding of the analogue to the T cell receptor is a specific binding. Generally during the binding discussed above the epitope or analogue is bound to an MHC class I molecule, such as HLA-A*0201.

The inhibition of binding can be determined using techniques known in the art or any of the techniques or under any of the conditions discussed herein. The T cell receptor used binds specifically to the epitope. T cells with such receptors can be produced by stimulating antigen naive T cells with any of the epitopes of the invention, for example using the stimulation protocol described in (1).

Typically an analogue is capable of causing antigen specific functional activation of a T cell which recognises the epitope (which can be measured using any of the techniques discussed herein). Generally the

analogue causes such activation when it is presented to the T cell associated with an MHC class I molecule, such as HLA-A*0201 (for example on the surface of a cell).

The analogue is typically capable of stimulating a MHC class I restricted T cell response directed to the epitope, for example when administered to a human or animal (such as in any of the forms or with any of the adjuvants mentioned herein). Such a response may be protective against a tumour challenge in an animal model or of therapeutic benefit in a human patient.

The analogue typically has a shape, size, flexibility or electronic configuration which is substantially similar to the peptide of the invention. It is typically a derivative of the peptide.

As well as binding the T cell receptor discussed above the analogue may also be able to bind other specific binding agents that bind the epitope. Such an agent may be HLA-A*0201. The analogue typically binds to antibodies specific for the peptide, and thus inhibits binding of the peptide to such an antibody. The analogue is either a peptide or non-peptide or may comprise both peptide and non-peptide portions. Such a peptide or peptide portion may have homology with the peptide of the invention.

The analogue may be at least 30% homologous to the peptide, preferably at least 50, 70, 80 or 90% and more preferably at least 95%, 97% or 99% homologous thereto, for example over a region of at least 20, preferably at least 30, for instance at least 40, 60 or 100 or more contiguous amino acids. Methods of measuring protein homology are well known in the art and it will be understood by those skilled in the art that in the present context, homology is calculated on the basis of amino acid identity (sometimes referred to as "hard homology"). For example the UWGCG Package provides the

BESTFIT program which can be used (e.g. on its default setting) to calculate homology (2).

The homologous peptide typically differs from the epitope by substitution, insertion or deletion, for example from 1, 2, 3, 4 or more substitutions and/or 1, 2, 3, 4 or more deletions and/or 1, 2, 3, 4 or more insertions over its length. The substitutions are preferably 'conservative'. These are defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

Typically the amino acids in an analogue which has homology with the peptide which are equivalent to amino acids in the epitope sequence (such as those which contribute to binding the MHC molecule or are responsible for the recognition by the T cell receptor) are the same or are conservatively substituted.

Typically in the analogue the amino acid in position 2 (based on the numbering used for epitopes bound to MHC molecules) is a L or M and/or the amino acid in position 6 is a V and/or the amino acid in position 9 is V or L.

The analogue (including the homologous peptide discussed above) may comprise 1, 2, 3, 4 more non-natural amino acids, for example amino acids with a side chain different from natural amino acids. Generally, the non-

natural amino acid will have an N terminus and/or a C terminus. The non-natural amino acid may be an L-amino acid.

Typically the analogue is a peptide which comprises one or more modifications. The sequence of the peptide may be the same as the epitope or homologous peptide discussed above. The modification may be any of the modifications mentioned above which can be present on the peptide of the invention.

The modification can be present on any of amino acids of the peptide, such as any of the the amino acids responsible for binding the MHC molecule or which contact the T cell receptor during recognition by a T cell.

The analogue is typically designed or selected by computational means and then synthesised using methods known in the art. Alternatively the analogue can be selected from a library of compounds. The library may comprise peptides which have an HLA-A*0201 binding motif. The library may be a combinatorial library or a display library, such as a phage display library. The library of compounds may be expressed in the display library in the form of being bound to a MHC class I molecule, such as HLA-A*0201.

Analogues can be selected from the library based on any of the characteristics mentioned above, such as their ability to mimic the binding characteristics of the original epitopes. Thus they may be selected based on ability to bind a T cell receptor, HLA-A*0201 or antibody which recognises the epitope. They may be selected based on their ability to cause antigen specific functional activity of a T cell that recognises the epitope (for example using any of the techniques or methods of the invention mentioned herein, e.g. CTL assays, ELISPOT assays or by measuring the production of cytokines inside the T cell).

The analogue which is capable of inhibiting the binding of the epitope to the T cell receptor after being processed is capable of being processed in the class I antigen processing pathway of a cell to provide a second analogue which can directly inhibit the binding of the epitope to the T cell receptor.

Such a cell is typically a mammalian or avian cell, such as a human or rodent (e.g. mouse or rat cell). The cell may be a muscle cell or a professional APC (such as a dendritic cell, macrophage, Langerhans cell or B cell). Typically the cell will provide the epitope or analogue on its surface bound to a MHC class I molecule.

The polynucleotide of the invention is capable of expressing 1, 2, 3 or more (different) compounds, each of which is (i) or (ii) (for example any combination of (i) or (ii) which is mentioned below in relation to (iv)). The polynucleotide is typically DNA or RNA, and is single or double stranded. The polynucleotide generally comprises 1, 2, 3 or more coding sequences which may be the same or different. At least one of the coding sequences encodes (i) or (ii). The coding sequence is typically operably linked to a control sequence capable of providing for expression of the polynucleotide. Thus typically the polynucleotide comprises 5' and 3' to the coding sequence sequences which aid expression, such as aiding transcription and/or translation of the coding sequence. Typically the polynucleotide comprises a promoter, enhancer, transcription terminator, polyadenylation signal, polyA tail, intron, translation initiation codon or translation stop codon.

The polynucleotide may be capable of expressing (i) or (ii) in a mammalian or avian cell, such as in any of the cells discussed herein. The polynucleotide may be capable of expressing (i) or (ii) in the cellular vector discussed below.

The polynucleotide may form or be part of a vector, such as a plasmid or cosmid vector. In one embodiment the polynucleotide is present in a virus or cellular vector, such as a virus which is capable of stimulating a MHC class I restricted T cell response (e.g. a vaccinia virus).

In the composition (iv) 1, 2, 3, 4, 5 or more different compounds may be present, wherein each of these compounds is (i), (ii) or (iii). Thus the composition may comprise all the epitopes of the invention (present in the form of the peptide of the invention), or instead of any of these epitopes the equivalent analogue. The composition may comprise 1, 2, 3, 4, 5 or more polynucleotides which together are capable of being expressed to provide 1, 2, 3, 4, 5 or more different epitopes, analogues or peptides of the invention, or all the epitopes (or instead of any of the epitopes the equivalent analogue) of the invention (e.g. in the form of peptide of the invention).

(i), (ii), (iii) or (iv) are provided for use in a method of vaccination against cancer or for use in a method of immunosuppression. 1, 2, 3, 4, 5, or more different epitopes of the invention (or all of the epitopes of the invention) may be used (or instead of any of these epitopes the equivalent analogue). As discussed above if more than one epitope/analogue is used then the combination of epitopes/analogues may be present in the form of the peptide of the invention or in the form of the composition of the invention. Similarly 1, 2, 3, 4, 5 or more different polynucleotides may be used which together are capable of being expressed to provide any of the combinations of epitopes, analogues, peptides or compositions mentioned herein.

However in one embodiment each epitope/analogue or one or more groups of epitopes/analogues within the

combination are administered to the host separately or sequentially. The epitopes/analogues in each group are typically together in the form of a single peptide of the invention or in the form of the composition of the invention. Similarly different peptides or polynucleotides of the invention may be administered separately or sequentially, for example polynucleotides capable of expressing individual or groups of epitopes and/or peptides and/or analogues and/or compositions.

Thus the invention provides a combination of 1, 2, 3, 4, 5 or more different epitopes and/or analogues and/or peptides and/or compositions and/or polynucleotides of the invention for simultaneous, separate or sequential use in a method of treatment of the human or animal body by therapy, for example in a method of vaccination against cancer or in a method of immunosuppression.

The method of vaccination against cancer or the method of immunosuppression typically leads to a MHC class I restricted T cell response, the T cells of which are specific for an epitope of the invention.

Thus (i), (ii), (iii), (iv) can be used in a form or manner in which they stimulate such a MHC class I restricted T cell response. Such methods are known in the art. Generally a MHC class I restricted T cell response can be obtained by vaccinating using an appropriate dose, route of administration, adjuvant or delivery system. Thus the vaccine of the invention may comprise one or more components (for example as discussed herein in relation to the vaccine of the invention) in addition to (i), (ii), (iii) or (iv). The components of the vaccine may be administered simultaneously, separately or sequentially to the host.

Thus the invention also provides a vaccine comprising (i), (ii), (iii) or (iv), which vaccine is

capable of stimulating a MHC class I restricted T cell response directed to an epitope of the invention. Typically such a vaccine comprises an adjuvant or delivery system which stimulates a MHC class I restricted T cell response.

The adjuvant may be capable of causing or augmenting a MHC class II restricted T cell (typically CD4) reponse which is favourable to the production of a MHC class I restricted T cell response, such as a Th1 response. Thus the adjuvant may comprise a MHC class II restricted T cell epitope (or a precursor which can be processed *in vivo* to provide such an epitope). The adjuvant may be a cytokine, such as a cytokine which stimulates a MHC class I restricted T cell response or favourable MHC class II restricted T cell response (e.g. IL-2, IL-7, IL-12 or IFN-γ). The adjuvant may be, for example, CFA (3), a muramyl dipeptide (e.g. of a mycobacterial cell wall), monophosphoryl lipid A, lipopolysaccharide (e.g. from B. abortus), liposomes, SAF-1 (3), a saponin (e.g. Quil A), keyhole limpet hemocyanin, yeast TY particle, beta 2-microglobulin or mannan (e.g. oxidised mannan).

The delivery system is typically capable of providing (i), (ii), (iv) or an epitope or analogue expressed from (iii) or (iv) to an APC, such as a professional APC.

As mentioned above the particular route of administration used may aid the stimulating of a MHC class I restricted T cell response, and thus (i), (ii), (iii), (iv) or the vaccine of the invention may be provided in a form suitable for administering by such a route. Intraperitoneal or intravenous routes are preferred. In one embodiment these substances are delivered by biolistic means.

Generally a low dose of antigen favours the development of a MHC class I restricted T cell response.

Thus in the method a suitable low dose of a compound of the invention can be given. The vaccine may be provided in an amount and concentration that is suitable for administering to provide an appropriate low dose.

In one embodiment (iv) is administered in the form of 'naked DNA'.

The T cell receptor of the invention recognises an epitope of the invention. The fragment of the T cell receptor typically comprises the extracellular domain. The fragment may be a soluble fragment or a fragment capable of binding to a cell membrane. The T cell receptor or fragment may be modified, such as by any of the modifications described herein in relation to the peptide of the invention. The T cell receptor or fragment may be part of a fusion protein.

The T cell receptor or fragment is able to bind an MHC molecule (e.g. HLA-A*0201) that comprises the epitope in its peptide binding groove. Typically the MHC molecule will be present on the surface of a cell. The T cell receptor or fragment may or may not be able to cause antigen specific functional activity of a T cell upon which they are present. This activity may include cytotoxic activity (such as the killing of the cell that bears the MHC/epitope complex which is recognised) or the secretion of substance (such as IFN-γ) from the T cell. The activity may be measured by CTL assay, ELISPOT assay or by measuring the production of cytokine inside the T cell.

The T cell receptor may be present in a population (or composition) which comprises 2, 3, 4, 5 or more different T cell receptors of the invention which together recognise any of the combinations of (i) or (ii) which are discussed above in relation to (iv).

The T cell of the invention comprises the T cell receptor of the invention. The T cell is a MHC class I

restricted cell, and is typically a CD8 T cell, although in one embodiment it is a MHC class I restricted CD4 cell. Generally when the T cell receptor of the T cell recognises the epitope antigen specific functional activity of the cell occurs (such as the functional activity mentioned above). The T cell may be an antigen naive or antigen experienced T cell. The T cell may be of a cell line, such as an immortalised cell line. The T cell may have been fused with another cell, which may or may not be a T cell.

The T cell is typically obtained from a host, such as a naive host, a host that has cancer or a host that has been immunised with a MUC1 based immunogen, such as any of the peptides, analogues or polynucleotides mentioned herein. The T cell may be replicated *in vitro* in an antigen specific (typically by contacting with an epitope or analogue of the invention) or a non-antigen specific manner. Thus the invention provides a T cell of the invention that has been produced by replication *in vitro*.

The invention also provides a product that selectively binds a T cell receptor of the invention, typically in a reversible manner. Such a product is generally able to inhibit the binding of an epitope of the invention (e.g. bound to an MHC molecule) to the T cell receptor. The product is typically able to cause antigen specific functional activity of a T cell with the T cell receptor of the invention.

The product typically comprises (a) an MHC molecule, or fragment thereof, comprising an epitope or analogue of the invention in its peptide binding groove, or (b) an analogue of (a) which is capable of inhibiting the binding of (a) to a T cell receptor of the invention.

The MHC molecule of (a) is generally a class I molecule (e.g. HLA-A*0201). Such molecules comprise an α

chain and a β chain. The fragment may comprise only the extracellular domain of the MHC molecule. The fragment may or may not be capable of binding a cell membrane.

(b) may comprise a protein which has homology with a naturally occurring α chain (or a fragment thereof) and/or a protein which has homology with a naturally occurring β chain (or a fragment thereof). The naturally occurring α or β chain may be of an HLA-A molecule (e.g. HLA-A*0201). Any of the above homologous proteins or fragments may be present as part of fusion proteins.

(b) is typically a derivative of (a), and thus may be made by modifying (a) by any of the modifications mentioned herein.

The product may be designed, made or identified using methods known in the art. Thus the invention provides use of an epitope or epitope sequence of the invention to design or identify the product. The product may be designed by computational means or may be identified from a library of compounds. Thus the invention provides a method of identifying a product of the invention comprising contacting a candidate substance with a T cell receptor or fragment of the invention and determining whether the candidate substance binds to the T cell receptor or fragment, the binding of the candidate substance to the T cell receptor or fragment indicating that the substance is such a product.

In the method the product may be present on the surface of a cell, such as a professional APC. The binding may be measured by contacting the candidate substance with a T cell of the invention and determining whether the candidate substance causes antigen specific functional activity of the T cell (such as by any means mentioned herein).

The product may be linked to a cytotoxic agent. In one embodiment the product is an antibody.

In one embodiment 2, 3, 4 or more products are linked together in a multimer, and thus the invention provides a multimer comprising 2 or more products of the invention. Such a multimer may be used in the same manner as the product is used in the different aspects of the invention, and thus the term 'product' as used in the context of the other aspects of the invention includes the multimer.

The products in the multimer may be linked by a covalent bond or by non-covalent means. In a preferred embodiment the products are linked by a streptavidin - biotin interaction, and thus typically the products comprise a biotin portion (typically chemically linked to or in a fusion protein with the product) which allows the products to be linked together by streptavidin.

The multimer generally has a higher binding affinity to the T cell receptor of the invention than the product, and in one embodiment is able to cause more antigen specific functional activity than the product. The multimer may also comprise a detectable label, such as a radioactive or a light detectable (e.g. fluorescent) label. The label may allow the multimer to be sorted by flow cytometry (e.g. when the multimer is bound to a T cell receptor which is present on a T cell of the invention).

The multimer may be a soluble multimer or may be capable of associating with a cell membrane. In one embodiment the multimer is attached to a solid support, such as a microtitre plate.

The invention also provides a cell comprising a product of the invention. The cell may be any of the types of cells mentioned herein, such as a professional APC or T cell. The cell may be capable of stimulating antigen specific functional activation of a T cell of the invention. Thus the cell may be used to stimulate a MHC

class I restricted T cell response *in vitro* or *in vivo*, which response is directed to an epitope of the invention. The cell may, therefore, be used in a method of treatment of the human or animal body by therapy, particularly in a method of treating or preventing cancer.

In one embodiment the cell may be made by providing (i), (ii), (iii) or (iv) to a cell which is able to process (i), (ii), (iii) or (iv) and present them on its surface (under conditions in which such processing occurs).

The invention provides a method of causing the replication of MHC class I restricted T cells which are specific for a cancer epitope comprising contacting a population of cells which comprises MHC class I restricted T cells with (i) or (ii) under conditions in which (i) or (ii) are presented to T cells in the population, or with a product or cell of the invention.

The invention includes use of a T cell of the invention (including a T cell replicated by the above method) *in vitro* or *in vivo* to kill a cell which presents the epitope of the invention. Such a cell is typically a cancer cell, but in one embodiment is a T cell (typically a MUC1 expressing activated T cell). Thus the invention provides a T cell of the invention, or a cell which has been replicated in the method of the invention for use in a method of treatment of the human or animal body by therapy. In particular for use in a method of preventing or treating cancer or a disease caused by an immune response, such as an inflammatory disorder, autoimmune disease, organ transplant rejection or graft versus host disease.

As mentioned above, the invention provides a method of identifying a MHC class I restricted T cell response which is based on determining whether MHC class I

restricted T cells from a host recognise (i), (ii)(either of which may be provided by (iii)), or a product or cell of the invention. In the method (i) or (ii) may be in the form of the composition (iv).

In one embodiment the determination of whether the T cells recognise (i) or (ii) is done by detecting a change in the state of the T cells in the presence of (i) or (ii) or determining whether the T cells bind (i) or (ii). The change in state is generally caused by antigen specific functional activity of the T cell after the T cell receptor binds (i) or (ii). Generally when binding the T cell receptor (i) or (ii) is bound to an MHC class I molecule, which is typically present on the surface of an APC.

The change in state of the T cell may be the start of or increase in the expression of a substance in the T cells and/or secretion of a substance from the T cell, such as a cytokine (e.g. IFN-γ, IL-2 or TNF-α). Determination of IFN-γ expression or secretion is particularly preferred. The substance can typically be detected by allowing it to bind to a specific binding agent and then measuring the presence of the specific binding agent/substance complex. The specific binding agent is typically an antibody, such as polyclonal or monoclonal antibodies. Antibodies to cytokines are commercially available, or can be made using standard techniques.

Typically the specific binding agent is immobilised on a solid support (and thus the method may based on the ELISPOT assay to detect secretion of the substance). After the substance is allowed to bind the solid support can optionally be washed to remove material which is not specifically bound to the agent. The agent/substance complex may be detected by using a second binding agent which will bind the complex. Typically the second agent

binds the substance at a site which is different from the site which binds the first agent. The second agent is preferably an antibody and is labelled directly or indirectly by a detectable label.

Thus the second agent may be detected by a third agent which is typically labelled directly or indirectly by a detectable label. For example the second agent may comprise a biotin moiety, allowing detection by a third agent which comprises a streptavidin moiety and typically alkaline phosphatase as a detectable label.

Alternatively the change in state of the T cell which can be measured may be the increase in the uptake of substances by the T cell, such as the uptake of thymidine. The change in state may be an increase in the size of the T cells, or proliferation of the T cells, or a change in cell surface markers on the T cell.

The change in state may be the killing (by the T cell) of a cell which presents (i), (ii) or the product of the invention to the T cell (e.g. the killing of the cell of the invention). Thus the determination of whether the T cells recognise the peptide may be carried out using a CTL assay.

In one embodiment the T cells which are contacted in the method are taken from the host in a blood sample, although other types of samples which contain T cells can be used. The sample may be added directly to the assay or may be processed first. Typically the processing may comprise diluting of the sample, for example with water or buffer. Typically the sample is diluted from 1.5 to 100 fold, for example 2 to 50 or 5 to 10 fold.

The processing may comprise separation of components of the sample. Typically mononuclear cells (MCs)are separated from the sample. The MCs will comprise the T cells and APCs. Thus in the method the APCs present in the separated MCs can present the peptide to the T cells.

In another embodiment only T cells, (in one embodiment only CD8 T cells), can be purified from the sample. PBMCs, MCs and T cells can be separated from the sample using techniques known in the art.

The T cells used in the assay can be in the form of unprocessed or diluted samples, or are freshly isolated T cells (such as in the form of freshly isolated MCs or PBMCs) which are used directly *ex vivo*, i.e. they are not cultured before being used in the method. However, more typically the T cells are cultured before use, for example in the presence of (i) or (ii), and generally also exogenous growth promoting cytokines. During culturing the (i) or (ii) are typically present on the surface of APCs, such as the APC used in the method. Pre-culturing of the T cells may lead to an increase in the sensitivity of the method.

The APC which is typically used in the method is from the same host as the T cell or from a different host. The APC can be an non-professional APC, but is typically a professional APC, such as any of the APCs mentioned herein. The APC maybe an artificial APC. The APC is a cell which is capable of presenting the peptide to a T cell. It is typically separated from the same sample as the T cell and is typically co-purified with the T cell. Thus the APC may be present in MCs or PBMCs. The APC is typically a freshly isolated *ex vivo* cell or a cultured cell. It may be in the form of a cell line, such as a short term or immortalised cell line. The APC may express empty MHC class I molecules on its surface.

In one embodiment the method identifies a MHC class I restricted T cell response to any of the combinations of (i) or (ii) discussed above in relation to (iv). Thus in the method the T cells can be placed into an assay with (iv) (which comprises the combination of (i) or (ii) which are to be tested. Alternatively the T cells can be

divided and placed into separate assays each of which contain a group of (i) or (ii) within the combination.

In one embodiment (i) or (ii) *per se* is added directly to an assay comprising T cells and APCs. As discussed above the T cells and APCs in such an assay could be in the form of MCs.

In one embodiment the (i) or (ii) are provided to the APC in the absence of the T cell. The APC is then provided to the T cell, typically after being allowed to present (i) or (ii) on its surface. (i) or (ii) may have been taken up inside the APC and presented, or simply be taken up onto the surface without entering inside the APC.

The duration for which the (i) or (ii) are contacted with the T cells will vary depending on the method used for determining recognition of the peptide. Typically the concentration of T cells used is 10^3/ml to 10^9/ml, preferably 10^5/ml to 10^7/ml. In the case where peptide is added directly to the assay its concentration is typically from 0.1 to 1000 μg/ml, preferably 10 to 100μg/ml.

Typically the length of time for which the T cells are incubated with (i) or (ii) is from 4 to 24 hours, preferably 6 to 16 hours.

The determination of the recognition of (i) or (ii) by the T cells may be done by measuring the binding of (i) or (ii) to the T cells. Typically T cells which bind the peptide can be sorted based on this binding, for example using a FACS machine. The presence of T cells which recognise the peptide will be deemed to occur if the frequency of cells sorted using the peptide is above a 'control' value (i.e. above the frequency of antigen naive T cells which recognise (i) or (ii)). The frequency of antigen-experienced T cells during a disease state can be up to 2.5% of the total CD8 T cells.

(i), (ii), (iii), (iv), the product or the cell of the invention can be used to detect a MHC class I restricted T cell response to an epitope of the invention *in vitro* (such as in a sample from a host) or in *vivo*. This can be done, for example, by using the method discussed above. The presence of a response generally indicates the presence of a cell which is expressing MUC1, such as a cancer cell or an activated T cell. Thus the detection of the response may be used to diagnose cancer. Measurement of the level of the reponse may be used to monitor the severity of the cancer (i.e. the number of cancer cells present in the host), a larger response indicating a more severe cancer.

In the method of diagnosis of the invention the presence or absence of the MHC class I restricted T cell response is typically determined by the method of identifying a MHC class I restricted T cell response discussed above.

The antibodies mentioned herein may be produced by raising antibody in a host animal. Such antibodies will be specific to the peptide or to the substances mentioned above which bind antibodies. The peptide or substances are referred to as the 'immunogen' below. Methods of producing monoclonal and polyclonal antibodies are well-known. A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the serum. The animal may therefore be inoculated with the immunogen, blood subsequently removed from the animal and the IgG fraction purified. A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells, for example as described in (4).

An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in vitro* immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat or mouse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled, for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

The inventors have also identified other epitopes which are represented by or present in the sequences SEQ ID NO:4, 5 and 6 (such as the fragments of the sequences shown in the brackets). These epitopes may also be used to provide peptides, analogues, polynucleotides, compositions, vaccines, T cell receptors, T cells, products and methods in the same way as the epitopes of SEQ ID NO's 1 to 3. Similarly such substances can also be used for vaccination against cancer or for immunosuppression.

SEQ ID NO:4 - SLSYTNPAV (SLSYTNPA or LSYTNPAV)
SEQ ID NO:5 - LLLTVLTVV (LLLTVLTV or LLTVLTVV)
SEQ ID NO:6 - ALGSTTPPA (LGSTTPPA)

Administration

Any of the peptides, analogues or polynucleotides discussed above in any form or in association with any

other agent discussed above is included in the termed 'vaccination agent' below. An effective non-toxic amount of such a vaccination agent may be given to a human or non-human patient in need thereof. The condition of a patient suffering from a cancer can therefore be improved by administration of such a vaccination agent. The vaccination agent may be administered prophylactically to an individual who does not have a cancer in order to prevent the individual developing cancer.

Thus the invention provides the vaccination agent for use in a method of treating the human or animal body by therapy. The invention provides the use of the vaccination agent in the manufacture of a medicament for vaccinating against cancer. Thus the invention provides a method of vaccinating an individual comprising administering the vaccination agent to the individual.

The vaccination agent is typically administered by any standard technique used for administering vaccines, such as by injection.

Typically after the initial administration of the vaccination agent a booster of the same or a different vaccination agent of the invention can be given. In one embodiment the the subject is given 1, 2, 3 or more separate adminstrations, each of which is separated by at least 12 hours, 1 day, 2, days, 7 days, 14 days, 1 month or more.

The vaccination agent may be in the form of a pharmaceutical composition which comprises the vaccination agent and a pharmaceutically acceptable carrier or diluent. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. Typically the composition is formulated for parenteral, intravenous, intramuscular, subcutaneous, transdermal, intradermal, oral, intranasal, intravaginal, or intrarectal administration.

The dose of vaccination may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. A physician will be able to determine the required route of administration and dosage for any particular patient. A suitable dose may however be from 10μg to 10g, for example from 100 μg to 1g of the vaccination agent. These values may represent the total amount administered in the complete treatment regimen or may represent each separate administration in the regimen.

In the case of vaccination agents which are polynucleotides transfection agents may also be administered to enhance the uptake of the polynucleotides by cells. Examples of suitable transfection agents include cationic agents (for example calcium phosphate and DEAE-dextran) and lipofectants (for example lipofectam™ and transfectam™).

When the vaccination agent is a polynucleotide which is in the form of a viral vector the amount of virus administered is in the range of from 10^4 to 10^{12} pfu, preferably from 10^7 to 10^{10} pfu (for example for adenoviral vectors), more preferably about 10^8 pfu for herpes viral vectors. A pox virus vector may also be used (e.g. vaccinia virus), typically at any of the above dosages. When injected, typically 1-2 ml of virus in a pharmaceutically acceptable suitable carrier or diluent is administered.

The invention is further illustrated by the accompanying drawing in which:

Figure 1 shows that MUC1 derived HLA-A*0201 binding peptides induce peptide specific cytotoxic CTL responses. $A2K^b$ mice were immunised twice with 100μg of MUC1 peptide in IFA and 140μg of Th peptide on day -28 and -14. On

day 0 single cell splenocyte suspensions were restimulated *in vitro* for one week with peptide loaded syngeneic LPS-elicited lymphoblasts and tested for cytotoxicity of peptide loaded Jurkat-A*0201K^b. Groups of A2K^b mice were immunised with MUC1 peptides MUC1$^{264-272}$ (FLSFHISNL), MUC1$^{460-468}$ (SLSYTNPAV), MUC1$^{13-21}$ (LLLTVLTVV), MUC1$^{167-175}$ (ALGSTAPPV) or MUC1$^{79-87}$ (TLAPATEPA). CTL bulk cultures were tested against Jurkat-A*0201K^b cells loaded with the cognate peptide (filled triangles) or irrelevant influenza matrix control peptide (open circles). Three representative graphs for each peptide are shown. The vertical axis shows % specific lysis.

The Examples also illustrate the invention:

Example 1

Prediction of peptides that bind HLA-A*0201

A computer program (5) was used to scan the MUC1 sequence with two tandem repeats for nine amino acid long peptides complying with the anchor residue motifs for HLA-A*0201. A full set of ninemers with an eight amino acid overlap covering the tandem repeat as well as ninemers in the top 10% of the scoring data for HLA-A*0201 were synthesised (90 peptides in total) by fmoc chemistry with a yield of 5-15 mg.

Example 2

Testing the synthesised peptides in a binding assay

Peptide binding to HLA-A*0201 was analysed using HLA-A*0201$^+$ B lymphoblastoid JY cells in a semi-quantitative competition assay (6). The assay is based on competitive binding of two peptides to acid stripped MHC class I molecules on a B cell line (JY). A test peptide competes with a fluorescently labelled reference peptide for the empty class I molecules on the cell surface. Mild-acid-treated JY cells were incubated with 150nM

fluorescein (FL)-labelled reference peptide FLPSDC(-FL)FPSV and with several concentrations of competitor peptide for 24 hours at 37°C in the presence of 1.0μg/ml β2-microglobulin. Subsequently, the cells were washed, fixed with paraformaldehyde and analysed by flow cytometry. The mean fluorescence (MF) obtained in the absence of competitor peptide was regarded as maximal binding and equated to 0%; the MF obtained without reference peptide was equated to 100% inhibition. The percentage inhibition was calculated using the formula:

{1-(MF 150nM reference and competitor peptide -MF no reference peptide)/(MF 150nM reference peptide - MF no reference peptide)}   x100%

The binding capacity of competitor peptides is expressed as the concentration needed to inhibit 50% of binding of the FL-labelled reference peptide (IC50). All peptides were tested several times in two fold dilutions starting with a concentration of 100μM. The six peptides that showed any significant binding were further analysed. The IC_{50} values of these peptides are shown in the table below together with the value for a flu peptide.

The peptides are defined in terms of the amino acid numbering used in Table 1. The tandem repeat can be defined using the restriction enzyme SmaI which cuts at CCCGGG three times in the MUC1 sequence, once either side of the tandem repeat and once in the C-terminus. This leads to the tandem repeat being defined as amino acids 129 to 148 in Table 1. The six peptides were analysed further as described below.

Peptide Position	Amino Acid Sequence	Motif Score *	IC_{50} mM/ml
Flu Matrix$^{58-66}$	GILGVVFTL	54	<5
MUC1$^{264-272}$	FLSFHISNL	59	3-5
MUC1$^{460-468}$	SLSYTNPAV	62	5-10
MUC1$^{13-21}$	LLLTVLTVV	63	6-10
MUC1$^{167-175}$	ALGSTAPPV	64	10
MUC1$^{79-87}$	TLAPATEPA	58	10-15
MUC1$^{107-115}$	ALGSTTPPA	56	25

* The algorithm used to define the motif score is described in (5).

Example 3

Testing the peptides in a cytotoxic T lymphocyte (CTL) assay

Summary of assay

To show that the six peptides were functional *in vivo*, transgenic mice expressing the chimeric protein A*0201K^b (7) underwent an immunization protocol with a MUC1-derived peptide and T helper epitope formulated with adjuvant. The mice were then sacrificed and the splenocytes restimulated by culturing with peptide-loaded, irradiated LPS-elicited B lymphoblasts. The restimulated cells were separated from the lymphoblasts and used in a CTL assay as effector cells. Effector cells were incubated with $Na^{51}CrO_4$ loaded target cells at various E:T ratios and cell killing estimated by measuring the amount of ^{51}Cr released into the cell supernatant using a gamma radiation counter.

Immunisation of mice with MUC1-derived peptides

Transgenic mice expressing the chimeric protein A*0201K^b (7) were immunised subcutaneously in the base of the tail with 100µg of MUC1-derived peptide and 140µg of H-2 I-A^b-restricted HBV core antigen-derived T helper epitope (amino acid sequence; TPPAYRPPNAPIL)(8) emulsified in a 1:1 ratio with Incomplete Freund's

Adjuvant (IFA) in a total volume of 200 µl. After a minimum of two weeks, the mice were boosted using the same protocol.

Preparation of LPS-elicited B lymphoblasts

Splenocytes from (unimmunized) transgenic mice expressing the chimeric protein A*0201K^b (7) were prepared 72h prior to use as stimulator cells. The cells of several mice were pooled and resuspended in IMDM N medium (IMDM (Biowhittaker) supplemented with 2mM L-glutamine, 8% (v/v) heat inactivated foetal calf serum (FCS), 20µM 2-mercaptoethanol and 100 IU/ml penicillin) containing 25µg/ml LPS (Sigma) and 7µg/ml dextran sulphate (Pharmacia). A 30 ml culture of cellular concentration, 1.5 X 10^6 cells per ml was incubated at 37°C for 72h.

Cells were then collected, resuspended in IMDM N, separated on a Ficoll gradient and adjusted to a cellular concentration of 5 x 10^6 cells/ml. Cells were then irradiated for 8 min (the equivalent of 2500 RAD). Cells were then washed once and resupsended in IMDM to a cellular concentration of 40 x 10^6 cells/ml.

Each MUC1 derived peptide, at a concentration of 100µg/ml, was incubated for 1h at 37°C with 1 ml LPS-elicited B lymphoblasts. The cells were then washed once and resuspended in IMDM N at a concentration of 10 x 10^6 cells/ml.

Restimulation of splenocytes from peptide-immunized mice

Two weeks after the final immunization, the mice were sacrificed and the spleens removed. Splenocytes (30 x 10^6 cells in a 9 ml volume of IMDM N medium were restimulated by incubation in complete medium with a 1ml volume of syngeneic, irradiated LPS-elicited B cell lymphoblasts (such that the ratio of splenocytes to blast

cells is 3:1). On day 7 of culture the cells were separated on a Ficoll gradient, resuspended in IMDM N medium and counted to generate a preparation of effector cells of known concentration.

Preparation of target cells

The Jurkat-A*0201K^b cell line which is a stable transfectant of a human T cell leukaemia line expressing the product of the HLA-A*0201K^b chimeric gene construct was used as a source of target cells.

Cells growing in log phase were harvested, washed once, counted and 10^6 cells transferred to a microfuge tube. The cells were pelleted and resuspended in a 100μl volume of 1 mCi/ml $Na^{51}CrO_4$ solution (Amersham) followed immediately by the addition of a 5μl volume of 1M HEPES pH 7.0 and gentle mixing of the cell suspension by pipetting. The tubes were incubated for 1h at 37°C. The cells were then washed four times in IMDM N medium and resuspended in a 25 ml volume of IMDM N medium containing the relevant peptide. After a 20 min incubation the cells were plated out into wells already containing effector CTLs. The final concentration of peptide in each well was 2μg/ml.

^{51}Cr release assay

Effector cells, prepared as above, were added in triplicate to wells of a 96 well plate (round bottom wells) such that the resulting ratio of Effector:Target cells was a range from 5:1 to 100:1. For each target cell line tested, six wells containing IMDM N or PBS with 2% (v/v) triton X-100 were prepared as controls to measure the spontaneous and maximal release of ^{51}Cr respectively.

A 50μl volume of the preparation of target cells (1000 or 2000 cells depending on preparation and number

of effector cells) was then added to each well and the 96 well plates centrifuged for 2 min at 1200rpm. The plates were then incubated for 6 h at 37°C. The culture supernatants from each well were then harvested using Skaton harvesting frames according to the manufacturer's instructions and the ^{51}Cr in each supernatant measured using a Wallac gamma counter.

The data was presented as %age specific ^{51}Cr release which is defined as 100 x ([experimental cpm - spontaneous cpm]/[total cpm - spontaneous cpm]) where the experimental value was the average of three test wells, the spontaneous value, the average of six wells containing IMDM N and target cells and the total value is the average of six wells containing 2% (v/v) triton X-100 and target cells. Data is shown for peptides $MUC1^{79-87}$, $MUC1^{167-175}$, $MUC1^{264-272}$, $MUC1^{460-468}$ and $MUC1^{13-21}$ in Figure 1.

Example 4

Protection assay

Mice were inoculated subcutaneously with 10^5, $5x10^5$ and 10^6 B16-MUC1-A2K^b cells (a melanoma cell line constitutively expressing MUC1 and the chimeric gene product HLA-A*0201K^b). Tumour growth was observed 20 days post inoculation and continued until sacrifice of the animal. An inoculation of $5x10^5$ B16-MUC1-A2K^b was defined as the optimal dose for tumour challenge experiments.

To test whether the HLA-A*0201 binding peptides that were previously identified could protect A2K^b transgenic mice (7) against subsequent tumour challenge with B16-MUC1-A2K^b, groups of 6-8 animals were immunised with 100μg of peptide in IFA in the presence of 140μg of the H-2 I-A^b-restricted HBV core antigen-derived T helper epitope (128-140; sequence TPPAYRPPNAPIL)(8), on day -28, boosted on day -14 and challenged with $5x10^5$ B16-MUC1-

A2K^b cells on day 0. Control mice were given IFA or PBS. A measurable tumour was defined as having a volume greater than 36 mm^3.

Results from these experiments are shown in the tables below in the form of the percentage of mice surviving at a given day. For experiments 2 and 3 results using a vaccinia construct that expresses MUC1 (VV-MUC1) are also shown. In other experiments immunising with MUC1$^{167-175}$ and boosting with MUC1$^{79-87}$ or immunising with MUC1$^{79-87}$ and boosting with MUC1$^{167-175}$ gave a percentage survival of between 60 and 70% at day 30. Experiment 3 shows results from an experiment in which the mice were inoculated with 8x10^5 A2K^b dendritic cells (DC) which had been pulsed with the peptides.

Experiment 1

Day	0	21	22	23	26	32	34
IFA	100	38	0	0	0	0	0
MUC1$^{264-272}$	100	75	75	75	63	63	63
MUC1$^{167-175}$	100	63	63	63	63	63	63
MUC1$^{79-87}$	100	100	75	75	75	75	63
MUC1$^{460-468}$	100	25	25	25	25	25	25
MUC1$^{13-21}$	100	25	13	13	0	0	0
VV-MUC1	100	75	75	75	63	63	38

Experiment 2

Day	0	13	24	26	28	33	38	46	52	53
IFA	100	100	70	50	50	40	40	30	30	30
MUC1$^{264-272}$	100	100	100	88	88	75	75	63	63	63
MUC1$^{167-175}$	100	100	88	63	63	63	63	38	38	38
MUC1$^{79-87}$	100	100	100	100	100	88	88	75	75	75
MUC1$^{460-468}$	100	100	100	75	75	50	50	38	38	38
MUC1$^{13-21}$	100	100	50	25	25	25	25	25	25	25
VV-MUC1	100	100	90	80	80	80	80	60	60	60

Experiment 3

Day	0	15	21	24	27	32	39	40	42	45	72
PBS	100	89	56	11	11	11	11	11	11	11	0
DC + Flu Matrix$^{58-66}$	100	88	63	50	50	38	38	38	38	38	38
DC + MUC1$^{264-272}$	100	100	88	88	88	88	88	88	88	88	88
DC + MUC1$^{167-175}$	100	100	78	78	78	78	78	78	67	67	67
DC + MUC1$^{79-87}$	100	100	89	89	89	67	67	67	67	67	67
DC + MUC1$^{460-468}$	100	100	75	63	38	38	25	25	25	13	13
DC + MUC1$^{13-21}$	100	100	67	56	56	44	22	22	22	22	22

Table 1

Met Thr Pro Gly Thr Gln Ser Pro Phe Phe Leu Leu Leu Leu Leu Thr
1 5 10 15

Val Leu Thr Val Val Thr Gly Ser Gly His Ala Ser Ser Thr Pro Gly
20 25 30

Gly Glu Lys Glu Thr Ser Ala Thr Gln Arg Ser Ser Val Pro Ser Ser
35 40 45

Thr Glu Lys Asn Ala Val Ser Met Thr Ser Ser Val Leu Ser Ser His
50 55 60

Ser Pro Gly Ser Gly Ser Ser Thr Thr Gln Gly Gln Asp Val Thr Leu
65 70 75 80

Ala Pro Ala Thr Glu Pro Ala Ser Gly Ser Ala Ala Thr Trp Gly Gln
85 90 95

Asp Val Thr Ser Val Pro Val Thr Arg Pro Ala Leu Gly Ser Thr Thr
100 105 110

Pro Pro Ala His Asp Val Thr Ser Ala Pro Asp Asn Lys Pro Ala Pro
115 120 125

Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr
130 135 140

Arg Pro Ala Pro Gly Ser Thr Ala Pro Pro Ala His Gly Val Thr Ser
145 150 155 160

Ala Pro Asp Asn Arg Pro Ala Leu Gly Ser Thr Ala Pro Pro Val His
165 170 175

Asn Val Thr Ser Ala Ser Gly Ser Ala Ser Gly Ser Ala Ser Thr Leu
180 185 190

Val His Asn Gly Thr Ser Ala Arg Ala Thr Thr Thr Pro Ala Ser Lys
195 200 205

Ser Thr Pro Phe Ser Ile Pro Ser His His Ser Asp Thr Pro Thr Thr
210 215 220

Leu Ala Ser His Ser Thr Lys Thr Asp Ala Ser Ser Thr His His Ser
225 230 235 240

Thr Val Pro Pro Leu Thr Ser Ser Asn His Ser Thr Ser Pro Gln Leu
245 250 255

Ser Thr Gly Val Ser Phe Phe Phe Leu Ser Phe His Ile Ser Asn Leu
260 265 270

Gln Phe Asn Ser Ser Leu Glu Asp Pro Ser Thr Asp Tyr Tyr Gln Glu
275 280 285

Leu Gln Arg Asp Ile Ser Glu Met Phe Leu Gln Ile Tyr Lys Gln Gly
290 295 300

Gly Phe Leu Gly Leu Ser Asn Ile Lys Phe Arg Pro Gly Ser Val Val
305 310 315 320

Val Gln Leu Thr Leu Ala Phe Arg Glu Gly Thr Ile Asn Val His Asp
325 330 335

Val Glu Thr Gln Phe Asn Gln Tyr Lys Thr Glu Ala Ala Ser Arg Tyr
340 345 350

Asn Leu Thr Ile Ser Asp Val Ser Val Ser Asp Val Pro Phe Pro Phe
355 360 365

Ser Ala Gln Ser Gly Ala Gly Val Pro Gly Trp Gly Ile Ala Leu Leu
370 375 380

Val Leu Val Cys Val Leu Val Ala Leu Ala Ile Val Tyr Leu Ile Ala
385 390 395 400

Leu Ala Val Cys Gln Cys Arg Arg Lys Asn Tyr Gly Gln Leu Asp Ile
405 410 415

Phe Pro Ala Arg Asp Thr Tyr His Pro Met Ser Glu Tyr Pro Thr Tyr
420 425 430

His Thr His Gly Arg Tyr Val Pro Pro Ser Ser Thr Asp Arg Ser Pro
435 440 445

Tyr Glu Lys Val Ser Ala Gly Asn Gly Gly Ser Ser Leu Ser Tyr Thr
450 455 460

Asn Pro Ala Val Ala Ala Thr Ser Ala Asn Leu
465 470

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CLAIMS

1. An agent selected from (i) peptides that comprise a MHC class I restricted T cell epitope, the epitope being contained in or represented by SEQ ID NO:1, 2, or 3; excluding whole MUC1, (ii) analogues of peptides (i) which are capable of inhibiting the binding of the said epitope to a T cell receptor either by directly binding to the same T cell receptor or by binding to the same T cell receptor after being processed, and (iii) polynucleotides which are capable of being expressed to provide peptides (i) or analogues (ii).

2. An agent according to claim 1(i), 1(ii) or 1(iii) wherein the peptide has the same sequence as the epitope being contained in or represented by SEQ ID NO:1, 2, or 3.

3. A composition (iv) that comprises two or more different agents, wherein each of the agents is as defined in claim 1 or 2.

4. An agent or composition as defined in any of the preceding claims for use in a method of vaccination against cancer or for use in a method of immunosuppression.

5. A vaccine comprising an agent or composition as defined in any one of claims 1 to 3; which vaccine is capable of stimulating a MHC class I restricted T cell response directed to an epitope as defined in claim 1.

6. A vaccine according to claim 5 which comprises an adjuvant or a delivery system, which adjuvant or

delivery system stimulates a MHC class I restricted response.

7. A T cell receptor which recognises an epitope as defined in claim 1; or a fragment of said T cell receptor which can recognise the epitope.

8. A T cell comprising a T cell receptor as defined in claim 7.

9. A T cell according to claim 8 which has been produced by replication *in vitro*.

10. A product that selectively binds a T cell receptor as defined in claim 7.

11. A product according to claim 10 which product comprises (a) an HLA molecule, or a fragment thereof, comprising an epitope or analogue as defined in claim 1 in its peptide binding groove, or (b) an analogue of (a) which is capable of inhibiting the binding of (a) to a T cell receptor as defined in claim 7.

12. A method of identifying a product according to claim 10 or 11 comprising contacting a candidate substance with a T cell receptor or fragment according to claim 7 and determining whether the candidate substance binds to the T cell receptor or fragment, the binding of the candidate substance to the T cell receptor or fragment indicating that the candidate substance is such a product.

13. A cell comprising a product as defined in claim 10 or 11.

14. A method of identifying a MHC class I restricted T cell response, said method comprising contacting a population of cells comprising MHC class I restricted T cells with:
peptide (i) or analogue (ii) as defined in claim 1 under conditions suitable for the presentation of peptide (i) or analogue (ii) to the T cells, or
a product or cells as defined in claim 10, 11 or 13; and
determining whether the CD8 T cells recognise peptide (i), analogue (ii), the product or the cell, recognition by the T cells indicating the presence of a MHC class I restricted T cell response.

15. A method according to claim 14 in which the determination of the T cell recognition is done by detecting the expression of a substance by the T cells, the expression of the substance indicating that the T cells have recognised peptide (i), analogue (ii), the product or the cell.

16. A method according to claim 15 in which the substance which is detected is IFN-γ.

17. A method of diagnosing cancer in a host said method comprising determining the presence or absence in the host of a MHC class I restricted T cell response to an epitope as defined in claim 1, the presence of the MHC class I restricted T cell response indicating that the host has cancer.

18. A method according to claim 17 in which the presence or absence of the MHC class I restricted T cell response is determined by the method of any one of

claims 13 to 16.

19. A method of causing the replication of MHC class I restricted T cells which recognise an epitope of a cancer cell or an activated T cell, said method comprising contacting a population of cells which comprises MHC class I restricted T cells with peptide (i) or analogue (ii) as defined in claim 1 under conditions in which peptide (i) or analogue (ii) are presented to T cells in the population, or with a product or cell as defined in claim 10, 11 or 13.

20. A T cell according to claim 8 or 9, or a cell which has been replicated in the method of claim 19, for use in a method of treatment of the human or animal body by therapy.

21. A kit for carrying out a method according to any one of claims 13 to 19 comprising peptide (i), analogue (ii), polynucleotide(iii) or composition (iv) as defined in claim 1 and/or a product according to claim 10 or 11.

22. Peptide (i), analogue (ii), polynucleotide (iii), composition (iv), a vaccine, a T cell receptor or fragment, a T cell, product or cell as defined in any one of claims 1, 2, 3, 5 to 10 and 13 for use in a method of treatment of the human or animal body by therapy.

23. Method of preventing or treating a disease in a host comprising administering to the host peptide (i), analogue (ii), polynucleotide (iii), composition (iv), a vaccine, a T cell receptor or fragment, a T

cell, product or cell as defined in any one of claims 1, 2, 3, 5 to 10 and 13.

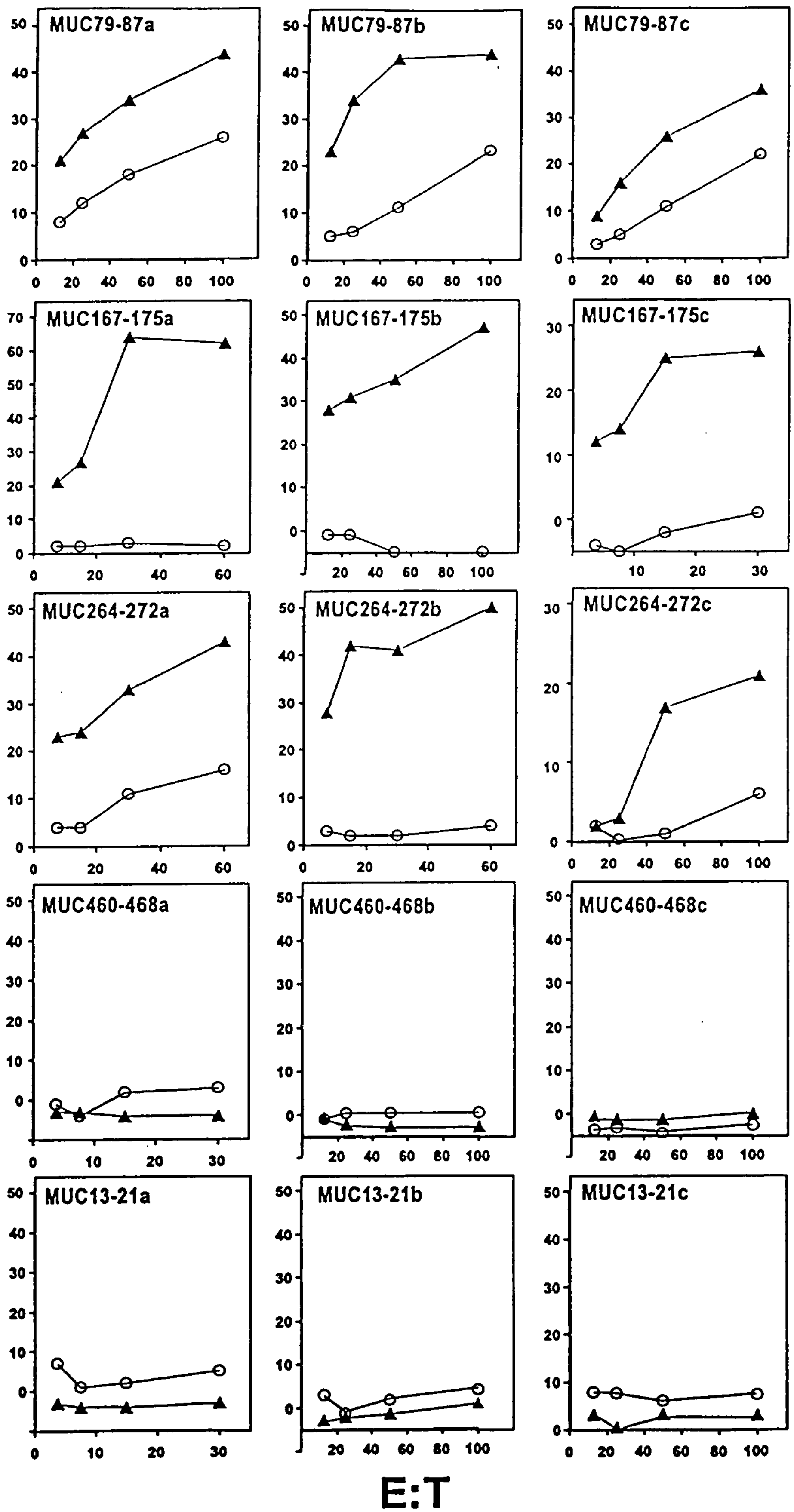

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FIG. 1